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Prospective Investigation of Cryptic Outbreaks of *Salmonella agona* Salmonellosis

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The number of *Salmonella agona* isolates reported annually in Texas from 1992 through 1994 ranged from 14 to 21. An increase in incidence of *S. agona* infections was noted in the fall of 1995. Pulsed-field gel electrophoresis (PFGE) analysis identified prospectively two possible cryptic outbreaks caused by an indistinguishable strain which was isolated from 18 of 59 patients who were culture positive from March through December 1995. These 18 patients had onset of illness from 20 May through 3 October 1995. Eight individuals resided in the Austin area, eight resided in San Antonio, and two resided in Houston; none had attended a common social gathering or owned common pets. Six patients in San Antonio and one patient from Houston recalled eating food items from the same Mexican food restaurant in San Antonio. *S. agona* organisms with the same PFGE profile were isolated from machacado, an air-dried, raw beef product prepared at the restaurant. The machacado had been shredded in a kitchen blender which was the probable source for cross-contamination of other food items. Five patients in Austin reported eating at a popular Mexican food restaurant in Austin. Improperly prepared machacado also may have been served at the Austin restaurant; however, sufficient quantities of machacado were not available for analysis. PFGE was essential in determining whether the cases constituted outbreaks and was invaluable in guiding the epidemiological investigation.

Pulsed-field gel electrophoresis (PFGE) has been applied to the study of the molecular epidemiology of numerous bacterial species (11, 13, 15). During outbreak investigations, PFGE has been utilized retrospectively to support the epidemiological data that associates the outbreak with a common source. PFGE frequently has been utilized to understand food-borne outbreaks of *Escherichia coli* O157:H7 diseases, shigellosis, listeriosis, and salmonellosis (1a, 2, 6, 8, 10, 12, 16).

In September 1995, an increase in the number of *Salmonella agona* isolates submitted to the Texas Department of Health (TDH) laboratory was recognized. We describe two outbreaks of *S. agona* salmonellosis in which PFGE was utilized prospectively to distinguish outbreak-related from non-outbreak-related strains and to guide the direction of the epidemiological investigation, which implicated an unusual source.

MATERIALS AND METHODS

Surveillance. Salmonellosis is a reportable condition in Texas. The annual number of cases and related demographic information were obtained from records maintained by the Infectious Disease Epidemiology and Surveillance Division, TDH. In September 1995, hospital laboratories in Texas were requested to submit *Salmonella* isolates to the TDH for serotyping. Laboratories that performed *Salmonella* serotyping were requested to submit *S. agona* isolates for PFGE testing at the TDH. *Salmonella* isolates were identified by routine laboratory procedures. Serotyping was performed by standard methods (Kauffmann-White scheme supplemented by the methods of the World Health Organization and the Institut Pasteur, Paris, France).

PFGE analysis. PFGE was performed as described elsewhere (11), with minor modifications. Plugs were cast with equal volumes of cell suspension and 2% low-melting-point agarose (Bio-Rad, Hercules, Calif.) and were lysed overnight at 55°C in a buffer containing 50 mM Tris, pH 8.0; 50 mM EDTA; 1% Sarkosyl; and 0.5 mg of proteinase K (Sigma, St. Louis, Mo.) per ml. DNA was digested overnight with *Xba*I (New England Biolabs, Beverly, Mass.). PFGE was performed by using 1.3% SeaKem Gold Agarose (FMC, Rockland, Maine) on a Bio-Rad CHEF Mapper with the following parameters: a two-state mode, a run time of 22 h 28 min at 6 V/cm, an initial switch time of 3.78 s, a final switch time of 75.1 s, and a ramping factor of 0.5. Image analysis of PFGE patterns was performed with an optical imaging system and RFLPScan software (Scanalytics, Billerica, Mass.). Interpretation of patterns was based on recommended guidelines for *S. agona* (15). Isolates were determined to be indistinguishable if they had the same number of restriction bands and the bands were of the same size. Isolates with a difference in only one band were considered similar. Isolates with more than one banding pattern divergence were considered different. The pattern for the outbreak strain was designated pattern A (also referred to as the outbreak pattern).

Case interviews. Patients were interviewed to ascertain demographic data, date of onset of illness, travel history, attendance at social gatherings, pet ownership, and names of restaurants visited during the three days prior to onset. Patients with a history of eating food items from a common restaurant in San Antonio were mailed the restaurant's menu. These restaurant patrons were reinterviewed by telephone and were asked to identify the specific food items that they usually ate at the restaurant. Mexican food restaurants in east Austin that served machacado were identified by contacting restaurant owners. Patients in Austin were reinterviewed to determine whether they had patronized specific Mexican food restaurants that served machacado and whether they had eaten machacado.

Restaurant inspection. Food samples from the restaurant in San Antonio associated with the cases were cultured for bacterial pathogens following protocols from the *FDA Bacteriological Analytical Manual* (1). Food preparation practices at the restaurants in Austin and San Antonio were reviewed with the owners by health department personnel.

RESULTS

From 1992 through 1994, 5,840 salmonellosis cases were reported in Texas. The number of cases reported annually ranged from 1,924 to 1,983. Only 30% of patients were 20 years of age or older, 43% were Hispanic, and 13% resided in San Antonio or Austin. The number of *S. agona* isolates reported annually in Texas from 1992 through 1994 ranged from 14 to 21, but from January through September 1995, 31 cases of *S.*

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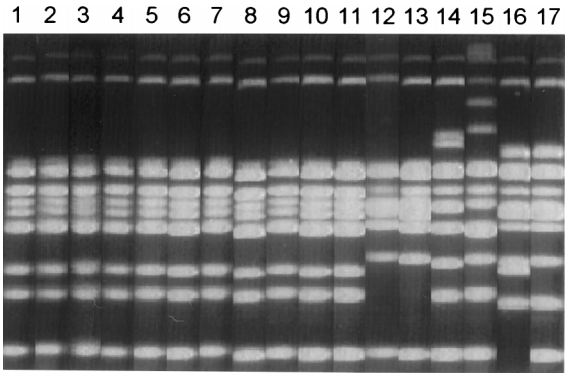

FIG. 1. *Xba*I PFGE patterns of 17 *S. agona* isolates. Lanes 1 to 11 show the outbreak strain.

agona infection were reported in Texas. Most of the 31 patients resided in central Texas: 13 patients resided in Austin and 10 resided in San Antonio (approximately 80 miles separate the two cities). The remaining eight patients resided in six cities throughout Texas. PFGE results on 17 central Texas isolates identified 11 (Fig. 1, lanes 1 through 11) that were indistinguishable; the pattern obtained for these isolates was designated pattern A. These initial isolates with pattern A were from patients who resided in the Austin area (seven patients) and San Antonio (four patients). Preliminary interviews with these patients did not identify a possible common exposure. Most patients were Hispanic (94%) and were 20 years of age or older (69%), dissimilar to the ethnicity and age groups generally associated with salmonellosis.

Surveillance efforts, which included notifying laboratories and local health departments of the possible outbreak, identified an additional 35 patients with *S. agona* infections. Isolates from 7 of these 35 patients were determined to have the outbreak pattern, increasing the total number of isolates with the outbreak pattern to 18. Of the 18 patients from whom the organisms were isolated, 8 resided in the Austin area, 8 resided in San Antonio, and 2 resided in Houston. These 18 patients had onset of illness from 20 May through 3 October 1995, and 11 experienced onset of illness after 1 August (Fig. 2). They had not attended a common church, school, or other social gathering, worked at a common location, or owned common pets. Most had difficulty recalling specific restaurants patronized 5 months prior to the interview.

One of the Houston residents had traveled outside the United States during the three days prior to onset of illness and had become ill while traveling. The second Houston resident had experienced onset of diarrhea on 22 July after returning from a 3-day trip to San Antonio. Because he had been there only briefly, he was able to recall specific restaurants he had patronized in San Antonio, unlike the patients who were San Antonio residents. San Antonio patients were reinterviewed to determine whether they had eaten at specific restaurants visited by the Houston patient. Six of the seven San Antonio patients and the Houston patient reported eating food items from one specific Mexican food restaurant in San Antonio. One patient was lost to follow-up. Patients reported eating a wide variety of food items from the restaurant. Because of

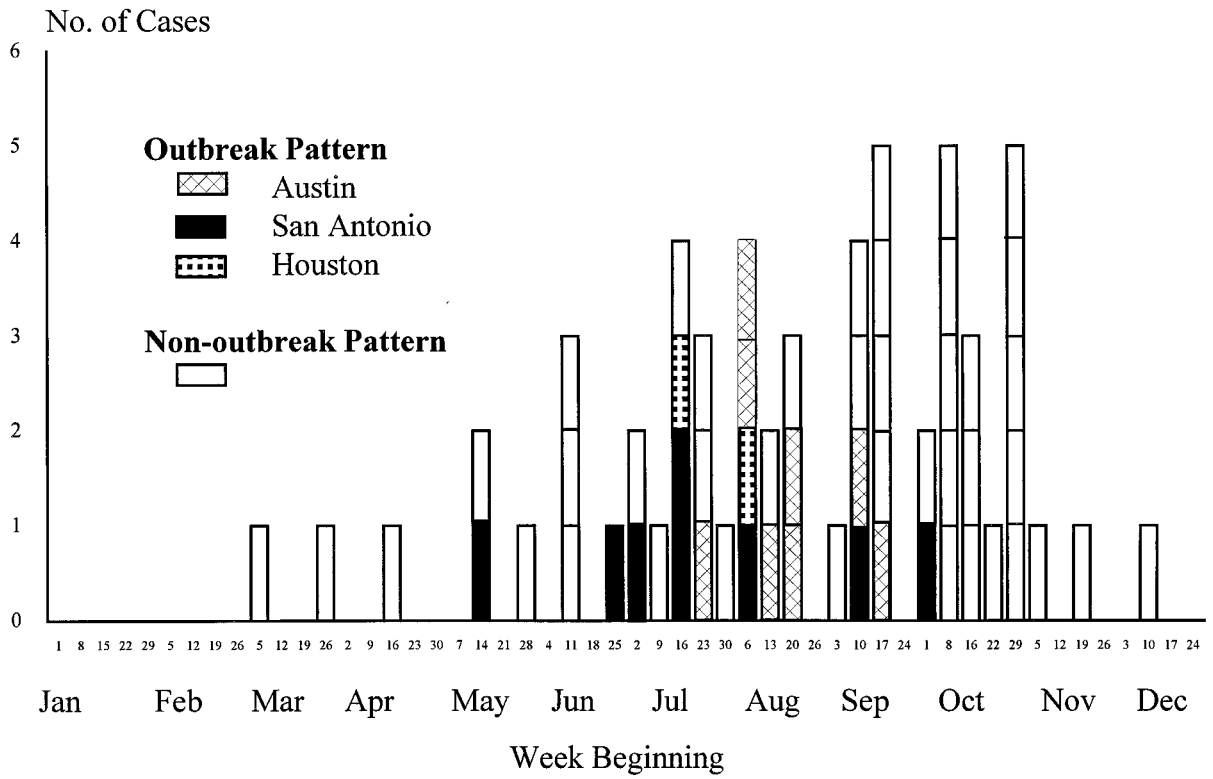

FIG. 2. Dates of onset of illness for patients with *S. agona* infections in Texas, January through December 1995. Patients with nonoutbreak PFGE patterns resided in 17 counties throughout Texas.

recall bias and the low number of cases, no analytical study was undertaken to implicate a specific food item.

Numerous food items, including raw eggs, guacamole dip, sausage, tripas (bovine stomach), barbacoa (cheek and head meat from a cow), and machacado (dried raw beef), were collected at the restaurant for bacteriological analysis. *S. agona* was identified only in the machacado. This isolate had a PFGE pattern indistinguishable from that of the seven isolates from patients with a history of eating at the San Antonio restaurant (data not shown). Strips of beef, without chemical preservatives, were hung to air dry without heat in the kitchen. The air-dried strips of beef were shredded in a kitchen blender and were then cooked and served with eggs or in a taco. This blender was also used to prepare an uncooked salsa served to all patrons in the restaurant. The blender was not routinely washed after preparation of the machacado. The restaurant was prohibited from preparing additional machacado. No additional salmonellosis cases were associated with the restaurant.

After the San Antonio source was identified, the Austin area patients were contacted to determine whether they had eaten at the implicated San Antonio restaurant, what Mexican food restaurants in Austin they patronized, and whether they ate machacado. Seven of the eight patients were reinterviewed; one patient was lost to follow-up. Although none had eaten food items from the San Antonio Mexican food restaurant, five reported eating at a popular Mexican food restaurant in Austin. The other two patients reported eating machacado: one made machacado at home, and the other purchased machacado from a street vendor. The owner of the Austin restaurant reported preparing machacado at his home. There were inconsistencies concerning whether the homemade machacado was served at the restaurant, but machacado was on the menu. Sufficient quantities of machacado from Austin for culturing were not available. Both the Austin and San Antonio restaurants purchased raw beef from a common distributor near Corpus Christi, Tex., but samples of raw beef from the same lot were not available.

A total of 66 patients with *S. agona* infections were reported in Texas in 1995. Isolates for PFGE were available for 59 patients (data not shown). The outbreak pattern was identified for 18 patients. The isolates from 34 patients had unique patterns. The isolates from seven patients had patterns judged similar to the outbreak pattern (the same number of restriction bands as the outbreak pattern but with one band of a different size). All seven patients were Hispanic, five were less than 2 years of age, and five resided well outside Austin and San Antonio. Two of these patients were lost to follow-up. These two patients were under 2 years of age and resided over 200 miles from Austin and San Antonio. None of the five available patients with isolates whose PFGE patterns were similar to the outbreak pattern reported a history of eating at either of the two restaurants; none ate machacado.

DISCUSSION

Two local outbreaks of salmonellosis caused by an apparently clonal strain of *S. agona* occurred in central Texas. These outbreaks involved two small groups of patients whose illnesses occurred sporadically over a 6-month period. These factors made recognition of the outbreak source in each city difficult. Although no analytical study was performed to provide a statistical association with the San Antonio restaurant, the presence of the indistinguishable organism in machacado at the restaurant was strong corroborative evidence that the restaurant was the source. Machacado, an air-dried, unpreserved, raw beef product, had not been linked previously to salmonellosis outbreaks, although other uncooked beef products had (3–5). The San Antonio outbreak was most likely caused by cross-contamination of products (e.g., salsa) prepared in an inadequately washed blender. This fact underscores the roles of improper food preparation practices and cross-contamination in salmonellosis outbreaks. A blender was also responsible for a mixed food-borne outbreak of salmonellosis and campylobacteriosis in a nursing home (9).

The evidence that machacado was the common source in Austin was less compelling. An adequate amount of machacado from the Austin restaurant was not available for testing. Two of the seven patients in Austin did not eat at the Austin restaurant, although both ate machacado from other sources. A common source for both outbreaks may have been the bulk beef utilized in preparing the machacado at the Austin and San Antonio restaurants. The bulk beef came from the same beef supplier in Texas, but additional beef samples of the same lot were not available. A single beef supplier in Denmark has been linked to a large outbreak of *Salmonella infantis* salmonellosis (16).

In 1991, the identification of a distinctive, antibiotic-resistant strain of *Salmonella typhimurium* was essential in the identification of a widespread, food-borne outbreak associated with uncooked ham (14). Similarly, differentiating between epidemiologically related and unrelated isolates by PFGE was essential in determining whether the Texas cases constituted outbreaks and in guiding the epidemiological investigation. The wide temporal and geographic distribution of cases hindered the recognition of the outbreak, and the two small outbreaks linked to machacado might not have been recognized among the 66 cases statewide without PFGE. The common source might never have been detected because most cases (70%) did not have the outbreak pattern. The association with the restaurant became clearer when patients with unrelated *S. agona* isolates were excluded. Other investigators have questioned the value of PFGE in routine surveillance (1a). These outbreaks demonstrated the value of utilizing PFGE for monitoring the emergence of a clonal strain associated with a common exposure because PFGE results for the 17 initial isolates indicated a possible common source for 11 patients.

S. agona strains in the general population have many different PFGE patterns. Of the 59 patient isolates typed, 34 had unique patterns. Indistinguishable patterns did not always imply a common exposure, as two patients whose isolates had the outbreak pattern did not have a history of consuming machacado or food items from either restaurant. One patient was most likely exposed outside the United States. Five of the seven patients with similar *S. agona* strains (one restriction band of different size) did not report exposure to the two restaurants. Restaurant exposure information for the two other patients with similar strains was unavailable, but they were infants who resided over 200 miles from either Austin or San Antonio and probably did not eat food items from the two restaurants. These data suggest that it is appropriate to exclude patients whose *S. agona* isolates differ from the outbreak restriction pattern only by band size from common-exposure investigations. Additional studies of common-source outbreaks may clarify these observations.

The outbreaks also demonstrated the value of disease surveillance. Recognition of the outbreaks was aided by the retrospective knowledge of the relatively low annual number of *S. agona* infections reported in Texas. The predominance of Hispanic patients and the geographical clustering of cases in Austin and San Antonio suggested a distinct exposure. Without recognition and intervention, additional restaurant patrons

could have been infected. Surveillance was also important in the recognition of other recent salmonellosis outbreaks (5, 7, 14). Our findings support the use of PFGE in monitoring the emergence of a clonal strain. Emergence may be recognized with only a few infected persons and may be related to a source amenable to control, thus preventing a larger or more serious outbreak.

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